# Mutagenic Activity of Disinfection By-Products

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Data on raw water quality, disinfection treatment practices, and the resulting mutagenic properties of the treated water were compiled from pilot- and full-scale treatment experiments to evaluate that parameter which might produce variability in the results of a mutagenic study.

Analysis of the data and comparison of treatment practices indicated that the measured mutagenic activity is strongly related to the characteristics of the organic matter in the raw water, the methodology used to sample and detect mutagens, the scale of the study both in terms of treatment flow and period of study, and the point at which and the conditions under which oxidants are added during treatment.

Conclusions regarding disinfection systems in full-scale water treatment plants include the following: When raw water is pretreated and high concentrations of organics are present in the raw water, both ozonation and chlorination increased mutagenic activity. However, no significant difference in mutagenicity was found between the two oxidants.

Both in the case of a nitrified groundwater and a clarified surface water, the mutagenic activity of the water after ozonation was related to its mutagenic activity before ozonation.

With ozonation, mutagenic activity decreased after granular activated carbon (GAC) filtration. Thus, when GAC filtration follows ozone disinfection, early addition of oxidants may not be deleterious to the finished water quality. When chlorine or chlorine dioxide is added after GAC filtration, chlorine dioxide was found to produce a less mutagenic water than chlorine.

Although these conclusions suggest means of controlling mutagenic activity during treatment, it must be stressed that the measurement of mutagenicity is a presumptive index of contamination level.

## Introduction

In 1975, Ames (1) described the correlation between mutagenic and carcinogenic activity: 90% of the carcinogenic compounds studied were found to be mutagenic and 87% of the noncarcinogenic compounds were found to be nonmutagenic. Since simple mutagenicity tests are used worldwide in several fields, especially in studies of water supplies, it would seem logical to evaluate the mutagenicity of compounds arising from disinfection processes. Previous work (2-5) in this area has focused on the effects of ozone and chlorine treatment. These chemicals have been shown to form mutagens when reacted with synthetic solutions representing raw waters (6-8). Other investigators have demonstrated the relationship between mutagenicity and surrogate parameters (e.g., ultraviolet absorbence) in the water disinfection process (3-8).

It is difficult, however, to relate the number of bacterial revertants per liter of water to a specific health risk as indicated by a given concentration of mutagenic material. Thus, a mutagenicity standard would be particularly difficult to establish.

Moreover, in complex mixtures, conflicting results of mutagenic activity have been observed. This paper demonstrates that results from mutagenicity tests are strongly influenced by the characteristics of the organic matter in the raw water, the methodology used to sample and detect mutagens, the scale of the study, both in terms of treatment flow and period of study, and the point at which and conditions under which the oxidants are added during treatment.

Following the discussion of the above issues, the problems and questions that arise when interpreting data associated with mutagenic activity of disinfection by-products are then reviewed.

## Material and Methods

### The Water Treatment Plants

The three water treatment plants that will be discussed are the Moulle plant, the Pecq-Medium plant, and the Vigneux pilot plant.

The Moulle water treatment plant (Fig. 1) is a 30-million gal/day (MGD) plant located near Dunkerque (northern France). Raw water with a dissolved organic carbon (DOC) content of 5–15 ppm of C is pumped from the Houlle river. Treatment involves prechlorination to breakpoint + 5 ppm of chlorine (Cl₂) followed by co-

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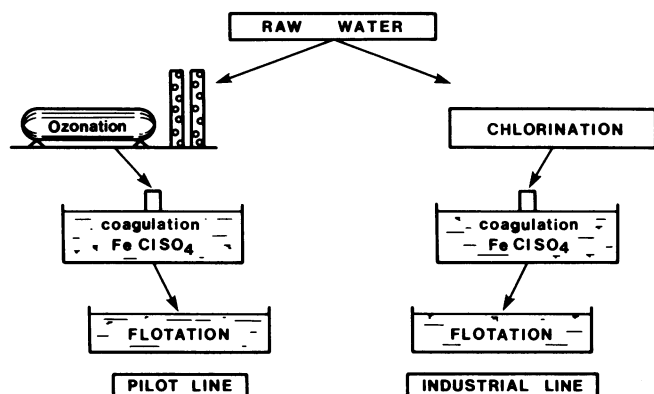†Laboratoire d'Hygiène de la Ville de Paris, 1 Bis rue des Hospitalières, St. Gervais 75004, Paris, France.

FIGURE 1. Diagram of the Moulle water treatment plant and pilot plant: preoxidation and clarification processes.

agulation (200 ppm ferric chlorosulfate), flotation, and granular activated carbon (GAC) filtration (Chemviron F 300). The finished water is used for groundwater recharge. A 50,000 gal/day (GPD) pilot plant was used to compare the effects of ozone versus chlorine as a pretreatment oxidant.

The Pecq-Medium water treatment plant (Fig. 2) is a 10-MGD plant located downstream of Paris, France. The 27 wells supplying this treatment plant are influenced to some degree by the recharging of the water table located nearby (12 to $13 \times 10^9$ GPD) for the 5 to 8 injected in groundwater recharge from clarified water from the Seine River. The DOC of the raw water varies from 1.5 to 2.5 ppm of C. The treatment involves removal of iron by aeration, biological nitrification, ozonation (1.5 ppm), GAC filtration (Norit ROW 08, EBCT: 15 min), and disinfection with chlorine (0.2 ppm). Nitrification is performed in vulcan ash filters to remove ammonia contained in the raw water.

The Vigneux pilot plant (Fig. 3) is located on the Seine River upstream from Paris. This 32,000-GPD pilot plant uses an upflow, solids-contact clarifier (pulsator, Degrémont, Rueil Malmaison, France) followed by rapid sand filtration (RSF). The filtered water (with a DOC of 1.5–2.5 ppm) is then distributed over four treatment lines to evaluate the efficiency of various ozone-GAC combinations. The GAC used in this study was a Calgon F 400 filter (Calgon Corp., Pittsburgh, PA). No regen-
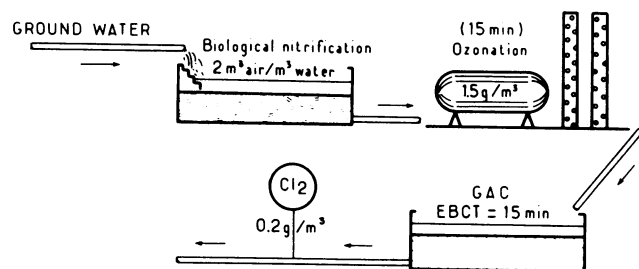


FIGURE 2. Diagram of the Pecq-Medium water treatment plant.

eration of GAC occurred during the survey. Final disinfection using chlorine ($Cl_2$) or chlorine dioxide ($ClO_2$) (0.2 ppm $Cl_2$) completes the process.

## Water Samples

Raw water quality varies dramatically with respect to micropollutants, both in concentration and composition. Thus, the collection of a representative sample becomes essential. For this reason, sample collection in this study was effected over a 2- to 4-day period, in which organics were extracted from 150–200 L of water (9). Although this method dilutes peak concentrations, the real concern is the chronic effect rather than the acute effect of micropollutants. Mutagenicity may result from several of the dissolved organics, thus the composite sampling procedure allows a representative background matrix more closely associated with chronic-type exposures to be collected.

The sample collector (Concentreur S656, SERES Co., Aix en Provence, France) enabled a composite sample to be collected over several days. Sampling is based on adsorption of organics on macroreticular resins (MRR) (XAD-2 and XAD-8, Rohm and Haas, Co., Philadelphia, PA). Water samples (100–200 liters) were contacted with a 100 mL bed of XAD-8 followed by a 100 mL bed of XAD-2 (10). The flow rate was set at 10 bed volumes per hour. The pH was adjusted to 2–3 with nitric acid. Sodium thiosulfate (0.1 N) was added to reduce any residual oxidant. Although the use of this reducing agent is known to decrease mutagenic activity, its addition was judged necessary to avoid oxidation of the resin and concentrated organics during sampling (11).

The adsorbed organics were eluted in the laboratory using dichloromethane (DCM) and methanol (MeOH). The first elution step with DCM produced an extract containing low molecular weight compounds that could be analyzed by gas chromatography (GC). The MRR was then eluted with MeOH to obtain an extract containing compounds that were more polar and/or that had higher molecular weights (10).

The solvents were then passed over anhydrous sodium sulfate to remove residual water and concentrated to 10 mL by Vigneux distillation evaporation. Further concentration to 3 mL was effected by evaporation under nitrogen.

The overall concentration factor from the water phase to final concentrate was between 30,000 and 60,000. One mL of each concentrate was assayed using the *Salmonella typhimurium* Ames test. The 1 mL of DCM was evaporated to dryness under nitrogen, and the organics were redissolved in dimethyl sulfoxide. MeOH extracts were concentrated to 1 mL and directly assayed in the Ames test.

## Ames Test

*Salmonella typhimurium* strains TA 98 and TA 100 were used according to the Ames test (1) to determine
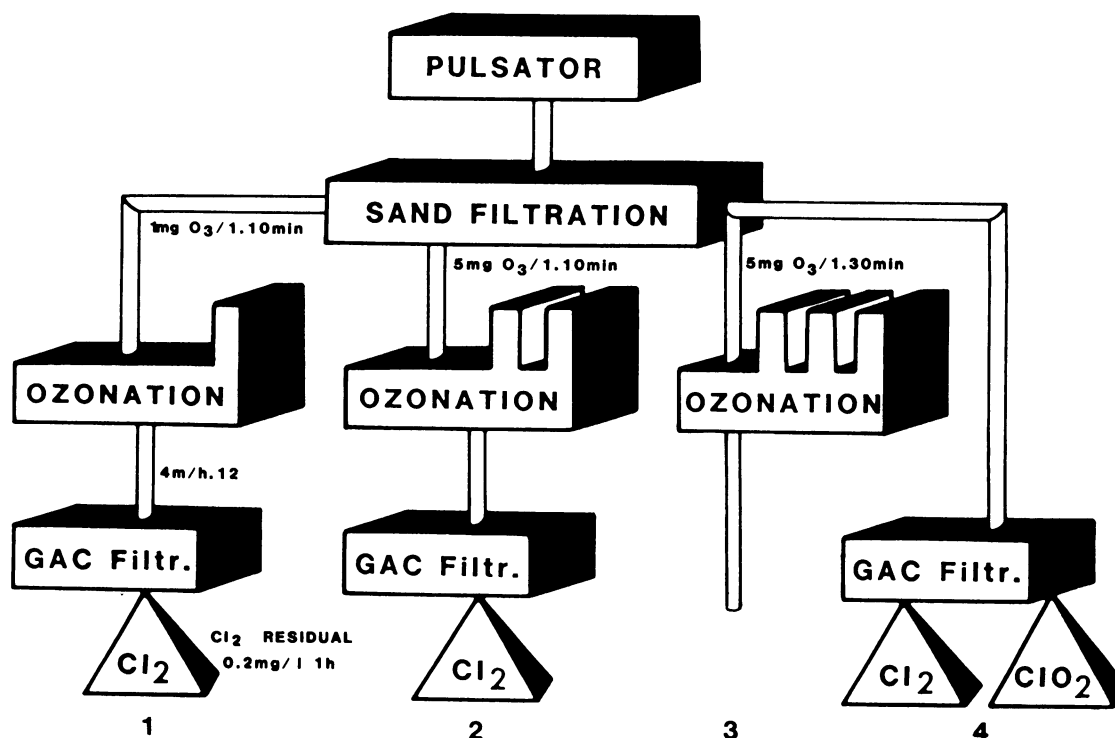
FIGURE 3. Diagram of the Vigneux pilot plant.

the mutagenic activity of the various water samples. DCM and MeOH or combined extracts were tested with and without S9 mix (microsomal fraction of induced Arochlor 1254 rat liver complemented with cofactors as described by Ames and co-workers). For each assay the number of revertants per plate was plotted versus the increasing volumes of water extracts tested. Then, the slope values determined by the linear regression of the dose-response curves were calculated for use in the statistical analysis.

Results were considered positive when a twofold increase of spontaneous revertants for at least one dose was observed and when a reproducible dose-response relationship was found. Results were considered uncertain when the first condition was not met and negative when neither condition was met.

Results using the TA 100 strain were often uncertain or negative for the first 4 months of the experiment at the Vigneux plant. After this time, the Ames test on this strain was no longer used.

## Statistical Methods

In the Vigneux pilot plant study, statistical methods were required to provide a quantitative interpretation of the mutagenicity data. Factorial analysis of correspondence and the Wilcoxon signed-rank test were used to quantify variations throughout the survey at the various sampling points.

## Wilcoxon Signed-rank Test

The Wilcoxon signed-rank test (*12*) is a nonparametric test that requires few assumptions about the underlying distribution of the data. In the Wilcoxon test, differences between paired values in two series of data are ranked in order of increasing absolute values. The sum of ranks derived from positive differences ($W+$) is then compared to the sum of negative ones ($W-$). Assuming there are no significant differences between the two series, the statistical distribution of $W(+)$ can be approximated by the Gauss-Laplace distribution. A bilateral test of probability is then applied to consider a positive or negative effect of the treatment. The critical probability (CP) then is calculated and compared to a threshold of 5%. When the CP is greater than 5% there is no significant difference between the 2 series (e.g., before and after treatment); otherwise the two series are considered to be significantly different.

## Factorial Analysis of Correspondence

In this method (*12–14*), mutagenicity values are grouped together in a matrix as shown in Figure 4.

In this representation, $k_{ij}$ is the mutagenicity value (slope of the dose response curve) for the month $i$ at the treatment point $j$. A summation of matrices from the various treatments (two types of bacteria TA 98/TA 100, two types of extracts DCM/MeOH, two types
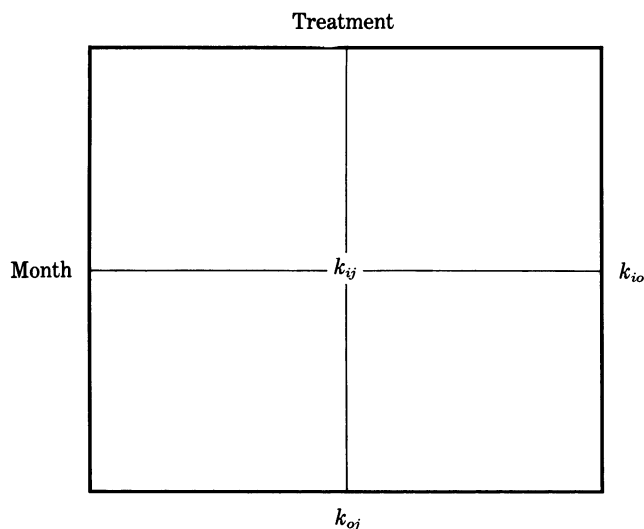
Treatment



FIGURE 4.  Mutagenicity matrix.



FIGURE 5.  Evolution of mutagenicity through preoxidation at Moulle water treatment plant and pilot plant (TA 98 without S9 mix).

of microsomal activation with S9 or without S9) is used in the statistical analysis.

The summation matrix can be considered as "clouds" of points in two spaces. One space (treatment space) would have a number of points equal to the number of rows, with the number of dimensions equal to the number of columns, and with the number of dimensions equal with the number of rows.

The statistical analysis determines the principal axes of inertia of the data point clouds. In a physical analogy, the axes of inertia of the solid defined by data points determine where the strongest relationships are, i.e., which mutagenicity values for a month are more strongly related to a treatment.

When a data set is well related, the first two or three eigenvalues (axes of inertia) account for 95% or more of the total inertia. In this study 80% to 95% of inertia is contained in the first three axes. This finding indicates that mutagenic activity can be related to treatment efficiency.

A two-dimensional projection of the clouds of data is then made with two axes of maximum inertia. In this plane, projected treatment points are clustered for each treatment.

## Results

Chemical oxidation in water treatment is conventionally performed at one or more of three steps in the disinfection process: during pretreatment, before GAC filtration, and during final disinfection. The results of tests on waters disinfected at each of these stages in the treatment process are presented below.
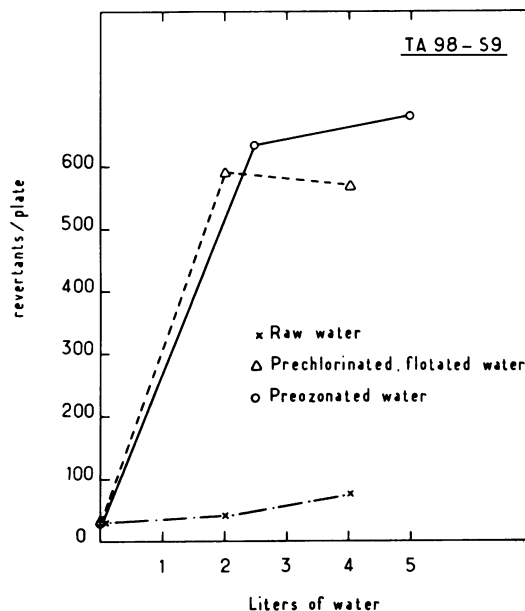
## Pretreatment: The Moulle Water Treatment Plant

Chlorine and ozone oxidizing agents are often applied to raw water when organic materials are present at high concentrations. This practice has been shown (15, 16) to produce toxic and mutagenic by-products. To promote the reduction or elimination of these by-products, we compared this conventional chlorine treatment method to alternative disinfection treatments, such as the ozonation process used at the Moulle treatment plant (Fig. 1).

At the Moulle plant, the raw water contains high concentrations of algae ($10^7$–$10^8$ algae/L), organic colloids (turbidity: 10–20 Nephelometric Turbidity Units), and DOC (5–15 mg/L). Chlorination induced formation of 100 to 200 µg/L of trihalomethanes (THM). The highest levels of mutagenicity of the DCM extracts were found using the TA 98 strain without metabolic activation (Fig. 5). Mutagenicity increased dramatically after oxidation and clarification. The type of oxidant used (either chlorine or ozone) did not significantly affect the mutagenic activity of the clarified water.

Chemical analysis of the DCM extracts was performed to help evaluate results of the mutagenicity tests. Many low molecular weight compounds were identified by gas chromatography-mass spectrometry (GC-MS) at low concentrations (Table 1). Ketones, alcohols, carboxylic acids, aldehydes, and chlorinated and nitrogenated compounds were among the most important compounds identified. Although most of these compounds were present in the raw water at undetectable concentrations (< 10 ng/L), chlorination produced significant concentrations of these compounds. Alkane ni-

**Table 1. Qualitative and semiqualitative results of the characterization of organic materials absorbed on resins from water at the Moulle Plant.**

| Compounds | RW, ng/L[a] | FIW, ng/L[a] | FPW, ng/L[a] |
|---|---|---|---|
| Chlorinated compounds | | | |
| Trichloroacetone | <10 | 10–50 | <10 |
| Tetrachloroacetone | <10 | 10–50 | <10 |
| Pentachloroacetone | <10 | 10–50 | <10 |
| Polychlorinated biphenyls | <10 | 10–50 | <10 |
| Carboxylic acids | | | |
| Benzoic acid | <10 | <10 | 5000–10000 |
| Dodecanoic acid | <10 | 500–2000 | 5000–10000 |
| Pentadecanoic acid | <10 | <10 | 2000–5000 |
| Hexadecanoic acid | 1000–2000 | <10 | 1000–2000 |
| Heptanoic acid | <10 | <10 | 20–200 |
| Octanoic acid | <10 | <10 | 20–200 |
| Ketones and alcohols | | | |
| Benzene ethanol | <10 | <10 | 50–100 |
| Acetophenone | <10 | 10–20 | 10–20 |
| Aldehydes | | | |
| Heptanal | <10 | <10 | 50–200 |
| Ethylbenzaldehyde | <10 | 10–20 | 10–20 |
| Nitrogenous compounds | | | |
| Butylbenzene sulfonamide | 20–200 | 500–1000 | <10 |

[a] RW: raw water; FIW: chlorinated and filtered industrial water; FPW: ozonated and filtered pilot plant water.



FIGURE 6. Evolution of mutagenicity through the Pecq-Medium water treatment plant (TA 98 with and without S9 mix).
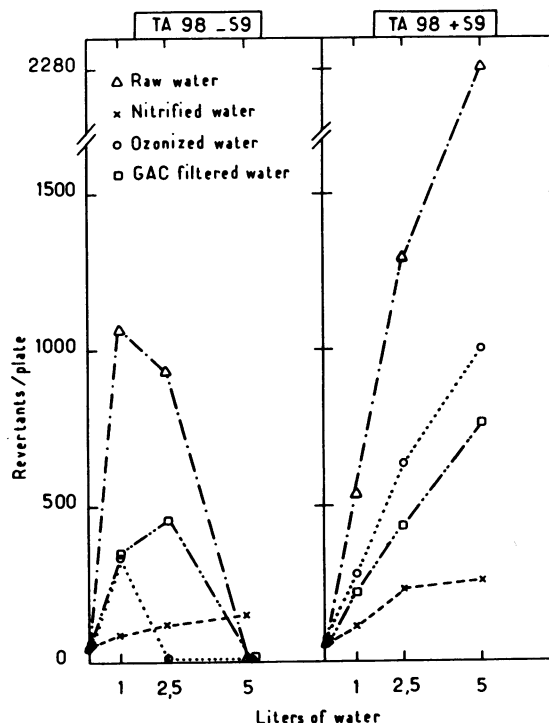
triles and chloropicrin have been similarly detected by using other analytical procedures (closed-loop stripping analysis). On the other hand, ozonation greatly increases the concentrations of carboxylic acids and other oxygenated organics.

More results must be compiled before the compounds responsible for the mutagenic activity are determined and before the significance of an equal level of mutagenicity produced by ozone or chlorine during pretreatment can be evaluated.

## Ozone–GAC Filtration Process: The Pecq-Medium Water Treatment Plant and the Vigneux Pilot Plant

In Europe, a combination of ozone and GAC filtration is often used to resolve problems such as poor taste, poor odor, disinfection, pollution spills, etc. The following sections provide results of analyses and tests on waters from plants using this type of process.

*The Pecq-Medium Water Treatment Plant.* MRR samples were collected at the Pecq-Medium plant from every process step (Fig. 2). Ames tests were performed on DCM-MeOH eluates from XAD resins. In the example given in Figure 6, the presence of a substantial quantity of mutagenic substance in the raw water can be seen. These substances appear to be removed effectively during the biological nitrification process, whereas ozonation leads to the formation of new mutagenic substances that in turn are eliminated by GAC filtration. However, in other samples, mutagenicity following ozonation was observed to either decrease or increase depending on the variations in the mutagenic activity of the nitrified water. The quality of the raw

water at any one time most likely depends on the wells in use at that time.

Forty compounds were identified by GC-MS at concentrations less than 100 ng/L, including phenols, alkylbenzenes, acetophenone, and the herbicide simazine. In view of the small concentrations involved, it is difficult to quantify reduction efficiency, but it is possible to state that the ozonation treatment results in a partial reduction of phenols and alkylbenzenes with the formation of aliphatic aldehydes.

Examination of surrogate parameters such as total organic halide (TOX) reveals that nitrification leads to an appreciable reduction in halogenated substances; however, sloughing effects at the outlet of the nitrification filters have been observed. In all cases, ozonation removes more than 50% of the TOX, and GAC filtration complements this removal.

Again, it is difficult to associate mutagenicity with identified compounds because it is not known which part of TOX is mutagenic and is extracted by MRR contactors. This question may be answered in more exhaustive chemical and biological analyses in long-term studies. The Vigneux pilot plant survey described below was designed to respond to some of these questions.

*The Vigneux Pilot Plant.* Chemical analysis (GC, GC-MS, DOC, etc.) and mutagenicity were examined over a 1-year period to evaluate combined ozonation/GAC processes, at several ozone doses and ozone contact times. Compounds identified by GC-MS and their concentration ranges at the different points (Fig. 3) of
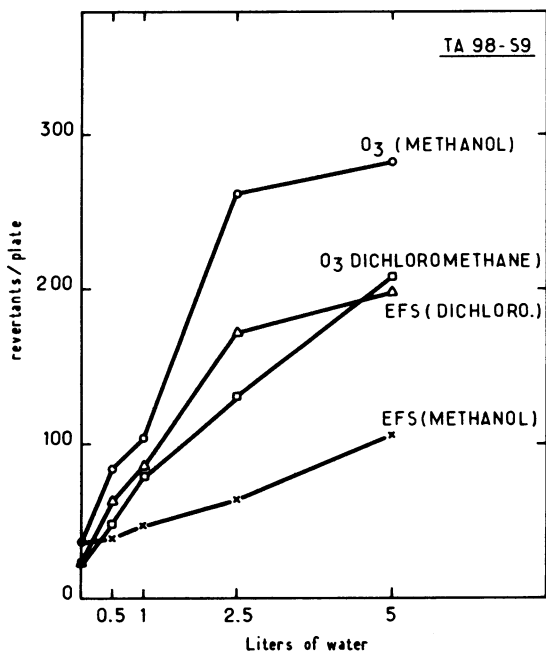
FIGURE 7. Evolution of mutagenicity with various ozonation conditions at Vigneux pilot plant (TA 98 without S9 mix, DCM extract). (RSF = rapid sand-filtered water).

Table 2. Evolution of mutagenicity with ozonation—Wilcoxon test analysis on TA 98 data (critical probability).[a]

| Ozone treatment | Extracts | | | |
| --- | --- | --- | --- | --- |
| | DCM | | MeOH | |
| | With S9 mix | Without S9 mix | With S9 mix | Without S9 mix |
| RSF/$O_3$ Line 1 | S (1.2%) | S (0.3%) | NS (84.9%) | NS (28.9%) |
| RSF/$O_3$ Line 2 | S (0.1%) | S (0.1%) | NS (25.8%) | NS (8.9%) |
| RSF/$O_3$ Line 3 | NS (69.7%) | NS (22.2%) | NS (86.5%) | NS (40.7%) |

[a] S = significant decrease of mutagenicity at 5% threshold; NS = no significant decrease of mutagenicity at 5% threshold; RSF = rapid sand-filtered water; critical probabilities (in %) are numbers in parentheses.

the pilot plant treatment process have been previously published (17, 18). The use of different sampling points allowed the various ozone treatments to be compared, alone or in combination with GAC (19).

Nine data sets were collected over the 1-year period. Graphs of mutagenicity values were drawn. Figure 7 shows the variations in mutagenicity with various ozonation rates. Variations of low mutagenicity values were observed during the period of the experiment. The ozone treatments represented by lines 1 and 2 reduced genotoxicity during the first 2 months of treatment and treatment at all ozonation rates reduced genotoxicity over 9 months of treatment. However, these observations were derived from the 36 mutagenicity values of 1 Ames data set (DCM extract, TA 98 strain, without S9 mixture). Interpretation of the other 682 values (a value is a slope as expressed in revertants per liter of water for a given sample point, either DCM or MeOH with a given strain, at a given time with or without S9 mix) resulted in conflicting conclusions. When simple graphic representation of Ames test data sets did not reveal any obvious trends, statistical methods were used to answer the following questions:

● What is the effect of ozonation on mutagenic activity?

● What effect did GAC filtration have on mutagenic activity? Did the combination of ozone and GAC result in any significant changes in mutagenic activity?

The following observations on ozonation were derived using the Wilcoxon signed-rank test, comparing mutagenic activity before and after ozonation over a 1-year period (Table 2). A significant decrease in mutagenic

activity was observed for ozone treatment lines 1 and 2 using the DCM extract. The highest ozonation rate changed mutagenic activity from that observed using the rapid sand filter. However, the MeOH extracts showed no statistical difference over the 1-year period.

Using the factorial analysis of correspondence method, the data can be displayed as shown in Figure 8. In this analysis, a trend is observed for the data clusters of MeOH and DCM extracts. These clusters become further removed from one another as the ozone dose increases. This result indicates that the difference between mutagenic activity in the two extracts (and
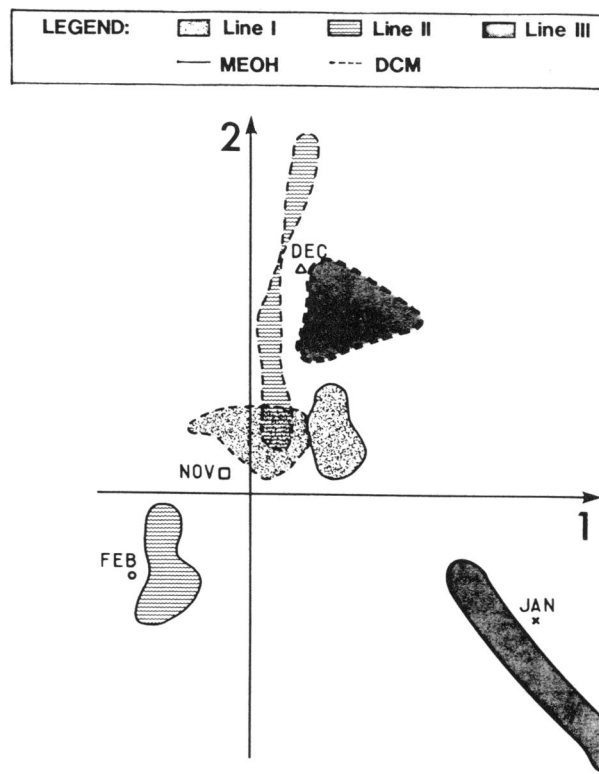


FIGURE 8. Factorial analysis of correspondence: evolution of mutagenicity after ozonation (first 4 months' data).

Table 3. Weighted classification of treatment line after ozonation for each month (from factorial analysis on TA 98 data).[a]

| Month | Treatment line | | | |
| | RSF-$O_3$ Line 1 | RSF-$O_3$ Line 2 | RSF-$O_3$ Line 3 | Relative ideal treatment |
|---|---|---|---|---|
| Sept., May | 3 | 1 | 2 | 3 |
| Jan., July, Dec. | 3 | 2 | 1 | 3 |
| Feb. | 2 | 1 | 3 | 3 |
| Nov., Mar., June | 1 | 2 | 3 | 3 |
| Sum of weights | 20 | 15 | 17 | 27 |
| % of ideal treatment line | 74% | 55% | 62% | 100% |

[a] RSF = rapid sand-filtered water.

Table 4. Evolution of mutagenicity with GAC filtration - Wilcoxon analysis on TA 98 data (critical probability).[a]

| GAC treatment | Extracts | | | |
| | DCM | | MeOH | |
| | With S9 mix | Without S9 mix | With S9 mix | Without S9 mix |
|---|---|---|---|---|
| $O_3$/GAC Line 1 | NS (32.2%) | NS (36.8%) | NS (39.5%) | NS (43.5%) |
| $O_3$/GAC Line 2 | NS (40.1%) | NS (9.3%) | NS (75.7%) | NS (29.8%) |
| $O_3$/GAC Line 4 | NS (45.9%) | NS (45.9%) | NS (96.0%) | NS (22.2%) |

[a] S = significant decrease of mutagenicity at 5% threshold; NS = no significant decrease of mutagenicity at 5% threshold; RSF = rapid sand-filtered water; critical probabilities in % are numbers in parentheses.

thus between the two types of compounds) is a function of the ozonation dose.

A second conclusion can be drawn when the distances between points representing each month are compared with the clusters of points representing mutagenicity of each ozone treatment. When the distance between points is short, the mutagenic activity value observed for the month at that treatment level is higher. Thus, the best treatment during a particular month in terms of mutagenicity, is represented by the cluster of points that is the farthest from the point representing the month under consideration.

These distances between months and each treatment level can be weighted to develop a hierarchic monthly classification of treatment efficiency. This classification of treatment efficiency is developed for each month and consists of weighting the distances between month and treatment clusters. A weight of 3 is attributed to the best treatment cluster (the cluster that is most distant from the month considered), a weight of 2 for the next most distant, and a weight of 1 for the least efficient treatment. Averaged over the year, the weights attributed to each treatment can be summarized to define which is the most efficient treatment. Then a relative, ideal, ozone treatment for minimizing mutagenic activity can be defined as treatment for which a weight of 3 is attributed for each month. The sum of the weights at one treatment level is used to determine a relative ideal ozone treatment. If one process produces the best results in each of the 9 months, it would have a score of $3 \times 9 = 27$, and would be 100% ideal ozone treatment. The ratios between the sum of the weights and the perfect scores thus determine the percentage of relative, ideal, ozone treatment. From this type of reasoning, ozone treatment line 1 is a 74% relative, ideal, ozone treatment and is more efficient than ozone treatment lines 2 and 3 in removing mutagenicity (Table 3).

The Wilcoxon signed-rank test was applied to compare the mutagenicity of samples before and after GAC filtration. Using data derived from DCM or MeOH extracts, the variations observed cannot be interpreted, and no statistical conclusions can be drawn. This is true for data from GAC treatment alone and for data from

all ozonation conditions (dose, contact time) with GAC filtration (Table 4).

Factorial analysis of correspondence was applied, integrating data both from DCM and MeOH extracts at the same time. Using the same interpretation here as that used for ozone treatment, the following conclusions are made for the 1-year study (Table 5). There is a difference between the ozone/GAC combination (line 1) and GAC filtration of clarified water. The ozone/GAC treatment yields an 89% relative, ideal treatment of the clarified water, whereas GAC alone yields 68%. The same difference is observed between the two ozone/GAC combinations (lines 1 and 2). Low ozonation rate treatment (lines 1 and 2) of this water source was observed to decrease mutagenic activity of low-molecular-weight and chromatographable compounds (DCM extract). Although GAC treatment seemed to change the composition of mutagenic activity qualitatively, no statistically significant changes were observed. However, in total, the ozone/GAC combination was more effective than GAC treatment without ozonation in decreasing mutagenic activity.

Conclusions that can be drawn from this survey are derived from the interpretation of small differences in low mutagenicity values, produced by low concentrations of organic materials. Relatively few organic compounds were identified in MRR extracts by GC-MS and

Table 5. Weighted classification of treatment lines after GAC for each month (from factorial analysis on TA 98 data).[a]

| Month | Treatment line | | | |
| | RSF-$O_3$-GAC Line 1 | RSF-$O_3$-GAC Line 2 | RSF-$O_3$-GAC Line 3 | Relative ideal treatment |
|---|---|---|---|---|
| Sept., Nov., May, Mar., June, Jan. | 3 | 2 | 2 | 3 |
| Feb. | 1 | 2 | 3 | 3 |
| July, Dec. | 3 | 2 | 2 | 3 |
| Sum of weights | 25 | 18 | 19 | 27 |
| % of ideal treatment line | 89% | 64% | 68% | 100% |

[a] RSF = rapid sand-filtered water.

**Table 6. Evolution of mutagenicity with disinfection—Wilcoxon test analysis on TA 98 data (critical probability).[a]**

| Disinfection treatment | Extracts | | | |
|---|---|---|---|---|
| | DCM | | MeOH | |
| | With S9 mix | Without S9 Mix | With S9 mix | Without S9 mix |
| GAC/Cl$_2$ | NS | NS | NS | NS |
| Line 1 | 83.4% | 26.7% | 58.9% | 55.5% |
| GAC/Cl$_2$ | NS | NS | NS | NS |
| Line 2 | 66.7% | 74.1% | 6.1% | 0.4% |
| GAC/Cl$_2$ | NS | NS | S | S |
| Line 4 | 92.8% | 66.0% | 2.1% | 0.7% |
| GAC/ClO$_2$ | NS | NS | NS | NS |
| Line 4 | 18.7% | 7.2% | 97.6% | 27.6% |
| Cl$_2$/ClO$_2$ | S | S | NS | NS |
| Line 4 | 3.3% | 0.3% | 17.6% | 44.7% |

[a] S = significant decrease of mutagenicity at 5% threshold; NS = no significant decrease of mutagenicity at 5% threshold; RSF = rapid sand-filtered water; critical probabilities (in %) are numbers in parentheses.

**Table 7. Evolution of mutagenicity with each treatment line Wilcoxon test analysis on TA 98 data (critical probability).[a]**

| Treatment Line | Extracts | | | |
|---|---|---|---|---|
| | DCM | | MeOH | |
| | With S9 mix | Without S9 Mix | With S9 mix | Without S9 mix |
| RSF/Cl$_2$ | S | NS | S | NS |
| Line 1 | 1.4% | 10.3% | 1.2% | 11.9% |
| RSF/Cl$_2$ | NS | NS | NS | NS |
| Line 2 | 8.9% | 63.8% | 23.9% | 15.0% |
| RSF/Cl$_2$ | NS | NS | S | NS |
| Line 4 | 10.7% | 31.7% | 0.9% | 16.5% |
| RSF/ClO$_2$ | S | S | NS | NS |
| Line 4 | 4.1% | 3.7% | 63.1% | 81.0% |

[a] S = significant decrease of mutagenicity at 5% threshold; NS = no significant decrease of mutagenicity at 5% threshold; RSF = rapid sand-filtered water; critical probabilities (in %) are numbers in parentheses.

direct-introduction MS. More disturbing is the presence of supposed MRR contaminants in the extracts. These compounds may be attributed to either the resins or the water sampled (acetophenone, alkylbenzenes, etc.). Cytotoxicity (measurement of RNA synthesis inhibition on *in vitro* cultured human cells) and TOX versus mutagenicity were analyzed by factorial analysis of correspondence and reveal no correlation with these parameters (20).

The Vigneux pilot plant survey demonstrates the complexity of mutagenicity behavior after ozonation-GAC treatment in natural waters at very low concentrations of organic materials.

## Final Disinfection: The Vigneux Pilot Plant

At the Vigneux pilot plant, such low levels of organic materials were present that the mutagenic activity produced by disinfection was difficult to establish. The Wilcoxon signed-rank test was used to compare the DCM extract before and after disinfection treatment, and no statistically significant differences could be attributed to disinfection with the exception of the MeOH extract, which showed a significant decrease in mutagenic activity for chlorine treatment line 2.

For the nonozonated GAC-filtered water, chlorine disinfection yielded greater mutagenic activity in the DCM extracts than did chlorine dioxide disinfection (Table 6).

Using factorial analysis of correspondence, the data on mutagenicity of water after disinfection was evaluated. Treatment lines were then compared (Table 7). Using the same interpretation (i.e., assigning weights of 3, 2, or 1) as for ozone and GAC treatment, the conclusions based on ordered classifications of treatment lines for each month and over a year are as follows: treatment line 1 is the best line associated with the pilot plant treatment and is a 92% relative, ideal, complete

treatment line; treatment line 2 is a 66% relative, ideal, complete treatment line; and treatment line 4 is a 41% line (Table 8).

These results suggest that disinfectants cannot be labeled mutagenic when they are applied to low levels of organics over long periods. Much of the time (41% to 92%), the level of mutagenicity of finished water was less than that of clarified water that was not prechlorinated.

## Discussion

In the results described above, ozone appears to either increase or decrease mutagenicity depending on two fields parameters: treatment conditions and the organic matter of the raw water.

An illustration of the influence of these parameters is presented in the study of the Pecq-Medium water treatment plant. A 12 mg O$_3$/min treatment rate was applied in a semibatch reactor to The Pecq-Medium raw water. Water samples from XAD resins were collected after 5, 10, 30, and 60 min of ozonation. DCM-MeOH extracts were evaluated using the TA 98 strain without metabolic activation. Results (Fig. 9) show that mutagenicity as a function of ozonation time is highly vari-

**Table 8. Weighted classification of complete treatment lines for each month (from factorial analysis of TA 98 data).[a]**

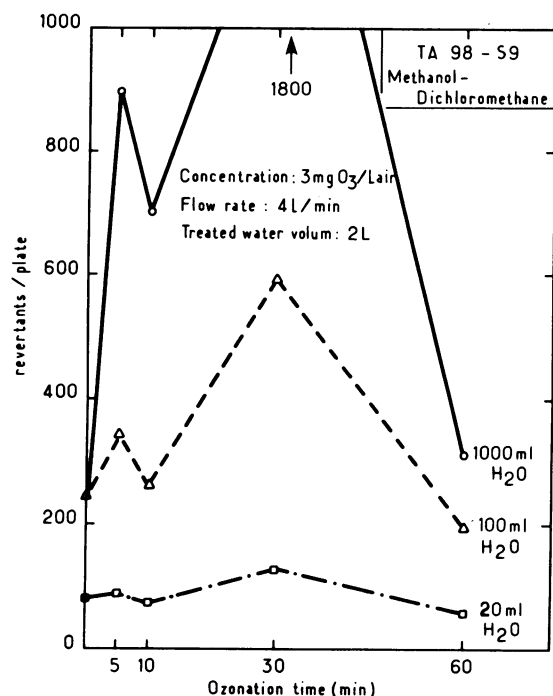| Month | Treatment line | | | |
|---|---|---|---|---|
| | RSF-GAC-O$_3$ Line 1 | RSF-GAC-O$_3$ Line 2 | RSF-GAC-Cl$_2$ Line 3 | Relative ideal treatment |
| Nov., Dec., May, June, July, Sept. | 3 | 2 | 1 | 3 |
| Jan. | 3 | 1 | 2 | 3 |
| Feb. | 1 | 3 | 2 | 3 |
| Sum of weights | 25 | 18 | 11 | 27 |
| % of ideal treatment | 92% | 66% | 41% | 100% |

[a] RSF = rapid sand-filtered water.

FIGURE 9. Variations of mutagenicity versus ozonation time of the Pecq-Medium raw water for 20, 100, and 1000 mL of equivalent water volumes.



FIGURE 10. Mutagenicity observed in DCM and MeOH extracts before (RSF) and after ozonation of the Pecq-Medium raw water (TA 98 without S9 mix).

able. Maximum effect was detected after 30 min of ozonation. A 60 min ozonation produced the same level of mutagenicity as that present in the raw water. As in the Vigneux pilot plant study, this experiment demonstrates that variations of mutagenicity during ozonation can be attributed to transformation of organics into by-products that can be more or less stable. In other words, minimum mutagenicity is obtained under well-defined ozonation conditions, but the optimum treatment rate is dependent on the organic matter variations in the raw water.

The mutagenicity related to by-products extractable on MRR may differ according to the nature of the solvent eluates. Successive elutions with DCM and MeOH solvents reveal the effects of ozonation on the changes in the nature of the organics. This result has been verified in several cases in the Vigneux pilot plant study. Mutagenic activity (in TA 98 without activation, Fig. 10) of the clarified water is higher in the DCM extract than in the MeOH extract. After ozonation, the genotoxicity of the MeOH extract increases, whereas a weak decrease is observed in the DCM extract. One explanation could be that part of the DCM-extracted compounds is oxidized by ozone into more polar compounds that are more easily eluted by MeOH.

Based on this example, the use of MeOh extract in the Ames test would appear to merit further investigation. Moreover, it appears that successive elutions of DCM and MeOH solvents are useful for GC-MS identification of compounds. Most MRR-extracted compounds eluted by DCM can be analyzed by GC tech-
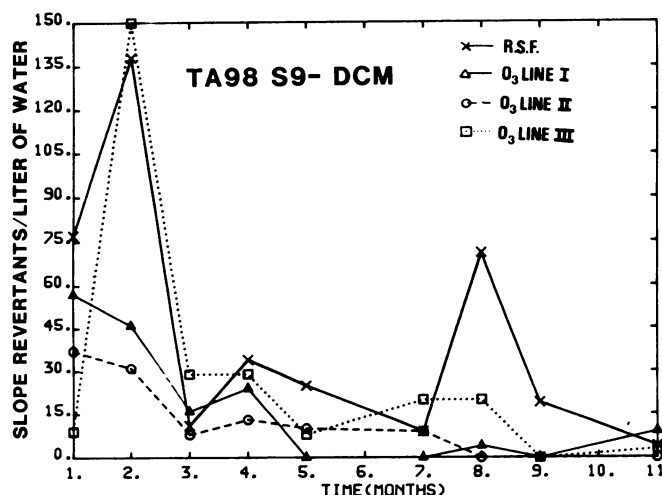
niques. On the other hand, GC analysis of compounds present in the MeOH extract has been found to be inefficient. To prevent the contamination of GC columns during injection of MeOH extracts, only DCM extracts were analyzed by GC and GC-MS. Other chromatography techniques such as high-pressure liquid chromatography (HPLC) were preferred for analyzing MeOH extracts. When separate DCM and MeOH extracts are analyzed, mutagenicity can be tested using DCM and MeOH extracts in combination or separately.

Adsorption techniques such as the MRR extraction process are subject to sloughing effects and competitive adsorption of organics. These phenomena can be dramatically affected by ozonation treatment. For example, mutagenic organics could be more easily extracted after ozonation (e.g., oxidation of competitive humic acid), and as a result, ozone would appear to be highly mutagenic.

Several authors (21) have shown that most of the compounds associated with health hazards and long-term health effects are lipophilic. Although they can be adsorbed by MRR and eluted by DCM, these compounds can be directly extracted by solvents such as DCM and chloroform. Therefore on-site, continuous MRR adsorption and liquid-liquid extraction of various samples must be performed and results compared (22). With regard to the sampling step, a recent experiment has shown that DCM extract of water (clarified with organic polymer coagulants) obtained by continuous liquid-liquid extractors were strictly mutagenic, whereas DCM-MeOH extracts from MRR contactors were primarly toxic and masked any mutagenic response in the Ames test.

All these results show that measurable mutagenic activity is significantly increased by oxidation when high levels of organics are present in the raw water. Thus, disinfection should be applied as late as possible in the

treatment line to reduce the formation of mutagenic by-products.

# Conclusions

Compounds responsible for genotoxic effects are difficult to identify in complex mixtures because of their low concentration, because of the variations in water quality, and because of the nature of disinfection by-products.

A sample to be tested must first be concentrated before its mutagenic activity can be determined since the compounds of concern are present at concentrations in the nanogram to microgram per liter range. Extraction techniques such as MRR may be used, but resin contaminants can affect mutagenic response; even if an analytical blank is used, we do not yet know the extent or the nature of these interferences in the mutagenicity test (synergism, antagonism, etc.).

To study mutagenicity, the methodology used should be standardized to allow comparisons among studies with minimum variation in the parameters. In fact, the organic matter of the waters may be so different (particularly before and after the ozonation process) that MRR columns and water volumes sampled would have to be predetermined for each sampling condition. In the adsorption technique, as with MRR sampling, breakthrough occurs in resin beds at specific times for each compound. Quantitative extraction techniques such as liquid-liquid extraction should be studied as alternative sampling techniques for subsequent mutagenicity tests.

From a treatment point of view, oxidation should be applied preferentially to waters containing low levels of organics to reduce or avoid the formation of mutagenic compounds. It is difficult to state that chlorine disinfection should be abandoned because of its mutagenic activity. Chlorine residuals may be necessary to eliminate microbiological problems, which, unlike mutagenic effects, are more easily quantified and perhaps more urgent.

In complex mixtures such as natural waters, it is difficult to connect the mutagenic activity exhibited by a water to the real risks incurred by consumers. It must be stressed that the measurement of mutagenicity is a presumptive index of contamination level. However, if extraction problems are resolved, genotoxicity testing enables various waters to be compared and allows various treatment processes to be evaluated qualitatively by this water quality parameter.

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